PTO CLAIMS/TJ

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1. (Amended) A method for detecting tumor-derived or tumor-associated RNA in the plasma or serum fraction of blood from a human or animal, wherein the tumor-derived or tumor-associated RNA is epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof, the method comprising the steps of:

a) extracting mammalian total RNA from plasma or serum, wherein a fraction of said extracted RNA comprises a tumor-derived or tumor-specific RNA species that is epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof;

b) amplifying or signal amplifying said fraction of the extracted RNA or corresponding cDNA prepared therefrom, wherein amplification is performed either qualitatively or quantitatively using primers or probes specific for the tumor-derived or tumor-associated RNA or cDNA corresponding thereto to produce an amplified product; and

c) detecting the amplified product produced from the RNA or cDNA.

2. (Amended) A method for detecting extracellular tumor-derived or tumor-associated RNA in a non-cellular fraction of a bodily fluid from a human or animal, wherein the tumor-derived or tumor-associated RNA is epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof, the method comprising the steps of:

a) extracting mammalian total RNA from a non-cellular fraction of a bodily fluid, wherein a fraction of said extracted RNA comprises an extracellular tumor-derived or tumor-specific RNA species that is epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof;

b) amplifying or signal amplifying said fraction of the extracted RNA or cDNA corresponding thereto, wherein amplification is performed either qualitatively or quantitatively using primers or probes specific for the tumor-derived or tumor-associated RNA or cDNA corresponding thereto to produce an amplified product; and

c) detecting the amplified product produced from the RNA or cDNA corresponding thereto.

3. The method of claim 1, wherein the amplification in step (b) is performed by a RNA amplification method that amplifies the RNA directly or wherein the RNA is first reverse transcribed to cDNA whereby the cDNA is amplified, and wherein the amplification method is reverse transcriptase polymerase chain reaction, ligase chain reaction, branched DNA signal amplification, amplifiable RNA reporters, Q-beta replication, transcription-based amplification, isothermal nucleic acid sequence-based amplification, self-sustained sequence replication assay, boomerang DNA amplification, strand displacement activation, or cycling probe technology.

4. The method of claim 2, wherein the amplification in step (b) is performed by a RNA amplification method that amplifies the RNA directly or wherein the RNA is first reverse transcribed to cDNA whereby the cDNA is amplified, and wherein the amplification method is reverse transcriptase polymerase chain reaction, ligase chain reaction, branched DNA signal amplification, amplifiable RNA reporters, Q-beta replication, transcription-based amplification, isothermal nucleic acid sequence-based amplification, self-sustained sequence replication assay, boomerang DNA amplification, strand displacement activation, or cycling probe technology.

5. The method of claim 1, wherein detection of the amplified product in step (c) is performed using a detection method that is gel electrophoresis, capillary electrophoresis, ELISA detection including using biotinylated or other modified primers, labeled fluorescent or chromagenic probes, laser-induced fluorescence, Southern blot analysis, Northern blot analysis, electroluminescence, reverse blot detection, or high-performance liquid chromatography.

6. The method of claim 2, wherein detection of the amplified product in step (c) is performed using a detection method that is gel electrophoresis, capillary electrophoresis, ELISA detection including using biotinylated or other modified primers, labeled fluorescent or chromagenic probes, laser-induced fluorescence, Southern blot analysis, Northern blot analysis, electroluminescence, reverse blot detection, or high-performance liquid chromatography.

7. The method of claim 1, wherein the RNA in step (a) is extracted from plasma or serum using an RNA extraction method that is a gelatin extraction method; silica, glass bead or diatom extraction method; guanidine-thiocyanate-phenol solution extraction method; guanidinium thiocyanate acid-based extraction method; phenol-chloroform-based extraction method; or involves centrifugation through a cesium chloride or similar gradient.

8. (Amended) The method of claim 2, wherein the RNA in step (a) is extracted from a non-cellular fraction of a bodily fluid using an RNA extraction method that is a gelatin extraction method; silica, glass bead, or diatom extraction method; guanidine-thiocyanate-phenol solution extraction method; guanidinium thiocyanate acid-based extraction method; phenol-chloroform-based extraction method; or involves centrifugation through a cesium chloride or similar gradient.

9. (Amended) The method for screening an animal or human for malignancy or premalignancy associated with epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof, the method comprising the steps of performing the method of claim 1 qualitatively or quantitatively, and detecting a product produced by said RNA in the plasma or serum of said animal or human, wherein detection of said RNA indicates that malignant or premalignant cells are present in the body of said animal or human.

10. (Amended) The method for screening an animal or human for malignancy or premalignancy associated with epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof, the method comprising the steps of performing the method of claim 2 qualitatively or quantitatively, and detecting a product produced by said RNA in the plasma or serum of said animal or human, wherein detection of said RNA indicates that malignant or premalignant cells are present in the body of said animal or human.

11. A method according to claim 9 wherein the animal is a human

12. A method according to claim 10 wherein the animal is a human

13. A method of identifying an animal or human having EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1 expressing cells or tissue, the method comprising the steps of:

a) extracting mammalian RNA from a bodily fluid of the animal or human;

b) amplifying a fraction of the extracted RNA or cDNA corresponding thereto, wherein said fraction comprises EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA, or hnRNP A2/B1 RNA or any combination thereof, and wherein amplification is performed qualitatively or quantitatively using primers specific for the RNA or cDNA corresponding thereto to produce an amplified product; and

c) detecting the amplified product produced from the RNA or cDNA corresponding thereto, whereby detection thereby identifies a human having EGF RNA, EGFr

RNA, her-2/neu RNA, c-myc RNA, or hnRNP A2/B1 RNA expressing cells or tissue.

14. The method of claim 13, wherein the EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA, or hnRNP A2/B1 RNA expressing cells or tissue comprise a malignancy, or premalignancy, or carcinoma in situ.

15. The method of claim 13, wherein the animal or human is one having a risk for developing a malignancy or premalignancy.

16. The method of claim 13, wherein the animal or human has been diagnosed as having a malignancy, premalignancy or carcinoma in situ.

17. A method for detecting or diagnosing a disease associated with expression of epidermal growth factor RNA, epidermal growth factor receptor RNA, her-2/neu RNA, c-myc RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 RNA in an animal, the method comprising the steps of detecting an amplified product according to claim 1 and detecting or diagnosing a disease associated with expression of epidermal growth factor RNA, epidermal growth factor receptor RNA, her-2/neu RNA, c-myc RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 RNA.

18. A method for detecting or diagnosing a disease associated with expression of epidermal growth factor RNA, epidermal growth factor receptor RNA, her-2/neu RNA, c-myc

RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 RNA in an animal, the metho comprising the steps of detecting an amplified product according to claim 2 and detecting or diagnosing a disease associated with epidermal growth factor RNA, epidermal growth factor receptor RNA, her-2/neu RNA, c-myc RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 RNA.

19. The method of claim 17 wherein the disease is a malignancy or premalignancy.

20. The method of claim 18 wherein the disease is a malignancy or premalignancy.

21. (Amended) A method for monitoring an animal or human for a malignant or premalignant disease, wherein the malignant or premalignant disease is associated with a tumor-derived or tumor-associated RNA that is epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 RNA, or any combination thereof, the method comprising the step of:

a) extracting mammalian total RNA from plasma or serum, wherein a fraction of said extracted RNA comprises epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof;

b) amplifying or signal amplifying said fraction of the extracted RNA or corresponding cDNA, wherein amplification is performed qualitatively or quantitatively using primers or probes specific for the tumor-derived or tumor-associated RNA or cDNA corresponding thereto, to produce an amplified product; and

c) detecting the amplified product produced from RNA or cDNA corresponding thereto.

22. A method for monitoring an animal or human for a malignant or premalignant disease, wherein the malignant or premalignant disease is associated with tumor-derived or tumor-associated RNA that is epidermal growth factor RNA, epidermal growth factor receptor RNA, her-2/neu RNA, c-myc RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof, the method comprising the step of:

1) detecting qualitatively or quantitatively RNA associated with the malignant or premalignant disease, wherein the RNA is epidermal growth factor RNA, epidermal growth factor receptor RNA, her-2/neu RNA, c-myc RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof, according to a method comprising the steps of:

a) extracting mammalian RNA from a bodily fluid, wherein a fraction of said extracted RNA comprises epidermal growth factor RNA, epidermal growth factor receptor RNA, her-2/neu RNA, c-myc RNA, heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof;

b) amplifying or signal amplifying said fraction of the extracted RNA or cDNA corresponding thereto, wherein amplification is performed qualitatively or quantitatively using primers specific for the tumor-derived or tumor-associated RNA or cDNA corresponding thereto, to produce an amplified product; and

c) detecting the amplified product produced from said RNA or cDNA corresponding thereto.

23. A method for selecting an animal or human with cancer for an epidermal growth factor-directed therapy comprising the step of performing the method of claim 1 using blood plasma or serum from said animal or human and selecting the animal or human for an epidermal growth factor-directed therapy when epidermal growth factor RNA is detected in the animal or human's plasma or serum.

24. A method for selecting an animal or human with cancer for an epidermal growth factor-directed therapy comprising the step of performing the method of claim 2 using a bodily fluid from said animal or human and selecting the animal or human for an epidermal growth factor-directed therapy when epidermal growth factor RNA is detected in the animal or human's plasma or serum.

25. A method for selecting an animal or human with cancer for an epidermal growth factor receptor-directed therapy comprising the step of performing the method of claim 1 using blood plasma or serum from said animal or human and selecting the animal or human for

an epidermal growth factor-directed therapy when epidermal growth factor receptor RNA is detected in the animal or human's plasma or serum.

26. A method for selecting an animal or human with cancer for an epidermal growth factor receptor-directed therapy comprising the step of performing the method of claim 2 using a bodily fluid from said animal or human and selecting the animal or human for an epidermal growth factor-directed therapy when epidermal growth factor receptor RNA is detected in the animal or human's plasma or serum.

27. A method for selecting an animal or human with cancer for a her-2/neu-directed therapy comprising the step of performing the method of claim 1 using blood plasma or serum from said animal or human and selecting the animal or human for an epidermal growth factor-directed therapy when her-2/neu RNA is detected in the animal or human's plasma or serum.

28. A method for selecting an animal or human with cancer for a her-2/neu-directed therapy comprising the step of performing the method of claim 2 using a bodily fluid from said animal or human and selecting the animal or human for an epidermal growth factor-directed therapy when her-2/neu RNA is detected in the animal or human's plasma or serum..

29. The method of claim 23 wherein the therapy is a monoclonal antibody or a tyrosine kinase inhibitor.

30. The method of claim 24 wherein the therapy is a monoclonal antibody or a tyrosine kinase inhibitor.

31. The method of claim 25 wherein the therapy is a monoclonal antibody or a tyrosine kinase inhibitor.

32. The method of claim 26 wherein the therapy is a monoclonal antibody or a tyrosine kinase inhibitor.

33. The method of claim 27 wherein the therapy is a monoclonal antibody or a tyrosine kinase inhibitor.

34. The method of claim 28 wherein the therapy is a monoclonal antibody or a tyrosine kinase inhibitor.

35. A method for selecting an animal or human with cancer for a cancer-directed therapy, the method comprising the steps of:

a) extracting mammalian RNA from plasma or serum of the animal or human, wherein a fraction of said extracted RNA comprises a tumor-derived or tumor-specific RNA that is epidermal growth factor RNA, epidermal growth factor receptor RNA, her-2/neu RNA, c-myc RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof;

b) amplifying or signal amplifying said fraction of the extracted RNA or cDNA corresponding thereto, wherein amplification is performed qualitatively or quantitatively using primers specific for the tumor-derived or tumor-associated RNA or cDNA corresponding thereto to produce an amplified product; and

c) detecting the amplified product produced from said RNA or cDNA, whereby detection thereof selects the human with cancer for a cancer directed therapy.

36. (Amended) A method for selecting an animal or human with cancer for a cancer-directed therapy, the method comprising the steps of:

a) extracting mammalian total RNA from plasma or serum of the animal or human, wherein a fraction of said extracted RNA comprises a tumor-derived or tumor-specific RNA that is epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof;

b) amplifying or signal amplifying said fraction of the extracted RNA or cDNA corresponding thereto, wherein amplification is performed qualitatively or quantitatively using primers or probes specific for the tumor-derived or tumor-associated RNA or cDNA corresponding thereto to produce an amplified product; and

c) detecting the amplified product produced from said RNA or cDNA, whereby detection thereof selects the human with cancer for a cancer directed therapy.

37. (Amended) A method according to claim 1, further comprising the step of performing a diagnostic test for diagnosing cancer or premalignancy when epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof is detected in plasma or serum of an animal or human.

38. The method of claim 35, wherein the cancer-directed therapy is surgery, chemotherapy, biologic therapy, vaccine therapy, anti-angiogenic therapy, or radiotherapy.

39. The method of claim 35, wherein the cancer-directed therapy is monoclonal antibody therapy or tyrosine kinase inhibitor therapy.

40. (Amended) A method for monitoring response to an anticancer therapy, comprising the step of performing the method of claim 1 on blood plasma or serum from an animal or human with cancer to whom anticancer therapy is administered, and wherein response to the anticancer therapy is accomplished by qualitative or quantitative detection of epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof.

41. (Amended) A method for monitoring response to an anticancer therapy, comprising the step of performing the method of claim 1 on blood plasma or serum from an animal or human with cancer to whom anticancer therapy is administered, and wherein response to the anticancer therapy is accomplished by qualitative or quantitative detection of epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, heterogeneous nuclear ribonucleoprotein A2/B1 RNA or any combination thereof.

42. A diagnostic kit comprising primers specific for amplifying epidermal growth factor RNA or cDNA prepared therefrom and reagents for extracting RNA from plasma or serum according to the method of claim 1.

43. A diagnostic kit comprising primers specific for amplifying epidermal growth factor receptor RNA or cDNA prepared therefrom and reagents for extracting RNA from plasma or serum according to the method of claim 1.

44. A diagnostic kit comprising primers specific for amplifying her-2/neu RNA or cDNA prepared therefrom and reagents for extracting RNA from plasma or serum according to the method of claim 1.

45. A diagnostic kit comprising primers specific for amplifying c-myc RNA or cDNA prepared therefrom and reagents for extracting RNA from plasma or serum according to the method of claim 1.

46. A diagnostic kit comprising primers specific for amplifying heterogeneous nuclear ribonucleoprotein A2/B1 RNA or cDNA prepared therefrom and reagents for extracting RNA from plasma or serum according to the method of claim 1.

47. A diagnostic kit comprising primers specific for amplifying epidermal growth factor RNA or cDNA prepared therefrom and reagents for extracting RNA from a bodily fluid according to the method of claim 2.

48. A diagnostic kit comprising primers specific for amplifying epidermal growth factor receptor RNA or cDNA prepared therefrom and reagents for extracting RNA from a bodily fluid according to the method of claim 2.

49. A diagnostic kit comprising primers specific for amplifying her-2/neu RNA or cDNA prepared therefrom and reagents for extracting RNA from a bodily fluid according to the method of claim 2.

50. A diagnostic kit comprising primers specific for amplifying c-myc RNA or cDNA prepared therefrom and reagents for extracting RNA from a bodily fluid according to the method of claim 2.

61. (Amended) A diagnostic kit comprising primers specific for amplifying heterogeneous nuclear ribonucleoprotein A2/B1 RNA or cDNA prepared therefrom and reagents for extracting total RNA from an acellular fraction of a bodily fluid according to the method of claim 2.

52. (Amended) A method for producing cDNA by reverse transcription of a fraction of extracellular mammalian total RNA extracted from plasma or serum, wherein the fraction comprises epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, heterogeneous nuclear ribonucleoprotein A2/B1 RNA, or any combination thereof, whereby cDNA corresponding to said RNA is produced.

53.(Amended) A method for producing cDNA by reverse transcription of a fraction of extracellular mammalian RNA extracted from an acellular fraction of a bodily fluid, wherein the fraction comprising epidermal growth factor RNA, epidermal growth factor receptor (erb-B-1) RNA, her-2/neu RNA, c-myc RNA, heterogeneous nuclear ribonucleoprotein A2/B1 RNA, or any combination thereof, whereby cDNA corresponding to said RNA is produced.